UNIVERSIDAD DE GUAYAQUIL
DEPARTMENT OF CHEMICAL SCIENCES

Ciudadela Universitaria ¨Dr. Salvador Allende¨

Telephone: 2293680, E-mail: fcquimic@ug.edu.ec

Guayaquil, Ecuador

FINAL REPORT

CODE: 17-05

TITLE:

Study of the acute oral toxicity of **Amantilla Relax**, originating from NUTRAMEDIX Laboratories, LLC, Florida, USA

OBJECTIVES:

To study adverse side effects produced by the administration of **Amantilla Relax** on body weight and different body systems.

BACKGROUND:

Amantilla Relax will be used in humans because of the vital importance of carrying out these first-step tests. They will not only guarantee the quality of the product, but will also establish that there are no adverse side effects in humans who take the product.

As discussed in numerous international works, the study of acute toxicity is indispensable, and guarantees (within the margin of error associated with the technique) that the potential for toxicity from the compounds that will be ingested or that may enter into the system accidentally will be learned.

Describing oral acute toxicity in the international literature is a requirement that must be fulfilled for all products that are to be introduced in the market for the first time. (1, 2, 3, 4, 5)

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SCIENTIFIC, TECHNICAL, AND SOCIOECONOMIC BENEFITS:

Demonstration of the innocuousness of this product is important in that the product could produce undesirable reactions in individuals who use it. Demonstrating that it does not produce toxic effects can lead to other tests that will allow it to be registered as a new medicine.

VARIABLES TO MEASURE:

- Toxic effects produced by oral application only of this product
- Weight of the animal's day 1, 7 and 14
- Mortality rates and time of death
- When clinical symptoms appear and disappear
- Anatomo-pathological exams (if required)

PROCEDURES TO FOLLOW:

Acute toxicity via oral introduction was determined using the procedures described in the OECD (Organization for Economic Cooperation and Development, comprised of the 24 most developed nations in the world) TG (Test Guidelines) 423.

CHANGES IN THE STUDY PLAN:

Changes did not take place in protocol proposed to the Unity of Quality Guarantee, whose number is referred to on Page 1**.**

SAMPLE DATA:

Product Name: **Amantilla Relax**

Represented by: Ing. José Icaza

Entity that carried out the work: University of Guayaquil, Department of Chemical Sciences

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Address: Ciudadela Universitaria ¨Dr. Salvador Allende¨
Represented by: Dr. Walter Herrera Arguello
Form of product presentation: glass bottle containing 30 ml
Storage: The product was stored at room temperature, was protected from light and kept under lock and key

INFORMATION WITH RESPECT TO HANDLING:
No special handling instructions were needed

PRODUCT COMPOSITION:
Valerian root extract
Mineral water
Ethanol (20 -25 %)

EXPERIMENTAL PROCEDURE:

INTRODUCTION:
This test was performed with the intention of determining the Acute Toxicity by oral intake of the product to be evaluated, given that this is one of the ways proposed for human intake.

DOSAGE USED IN THE TEST:
Data used indicates that:

Suggested use is 5 to 10 drops once a day. Additionally these drops are dissolved in a 120 ml glass of water. With this data in mind, each mouse received 20 ml/kg of body weight, but without dissolving in water.

Mortality rates and other clinical observations as are discussed in Table 1 were used as fundamental test parameters.

PRINCIPAL TEST PROCEDURE FOLLOWED:
Those that are described in the norms of the OECD. (5)

METHODS AND TECHNIQUES:

Study Material: Amantilla Relax

Animal Model: The test was carried out with a species of rodent (mouse), with a minimum of 6 animals per test of the same sex. That is, 6 females were used in accordance with recommendations with a mean weight of ±20% (7), and belonging to the Swiss line, and originating from the Chemistry Department of the University of Guayaquil. These mice were appropriate for carrying out the study of acute toxicity via oral intake.

The animals were maintained in climate-controlled and quarantine conditions according to established procedures (8, 9), during a period of at least 5 days.

Access to food and water was “ad libitum.¨(10, 11)

Animals were distributed randomly among the different groups. (12)

Food was denied the mice 18 hours before exposure to the test substance.

The test lasted 19 days (5 days of acclimation, 14 test days)

METHOD DEVELOPMENT:
Two experimental groups were created. Only females were used, as is in accordance with OECD guidelines 423 of 2001, asserting that the female is more sensitive than the male, making it a more appropriate choice for acute toxicity studies.

The evening before the experiment food was denied the animals with the testing carried out after this fasting. After the fasting all animals were weighed to determine the appropriate dosage.

The substance administered was the study product, in a single dosage of 20 ml/kg of animal weight. Two to three hours after the administration of the product the animals were allowed access to food again.

After the product's administration observations were conducted and systematically recorded for each individual animal, several times on the first day and at least once a day for the next 13 days.

Given that oral ingestion of the product could cause delayed toxic reactions, the animals were weighed on the first, seventh, and 14th days.

At the end of the experiment, the animals were euthanized in a saturated ether atmosphere. (13)

If any abnormality were detected during the examination of the organs (lungs, heart, kidneys and stomach or other organs that may have shown clinical symptoms during the clinical studies), samples were taken for pathological studies (14).

RESULTS CALCULATIONS:
The weights of the mice at different times were statistically processed to obtain the mean and the standard deviation (2).

DESCRIPTION OF THE DOSAGE, ADMINISTRATION METHOD AND DURATION OF THE EXPERIMENT:
The experiment was conducted following the guidelines of OECD TG 423.

The method of administration was oral, using an intra-gastric canella.

The experiment lasted 19 days (5 of acclimation and 14 of testing).

It is important to realize that this experiment was carried out using a volume of 20 ml per kilogram of body weight. In comparison, a human would be expected to ingest 10 drops, or approximately 0.3 ml dissolved in 120 ml of water. This means 0.0025 ml per 60 kg of body weight, or 0.00041 per kg of body weight. The mice receive 20 ml per kg of body weight, or 500000 times the expected human dosage.

ANALYTICAL RESULTS:
Results of the daily observations during the 14-day experimental period are recorded in Table 1.

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TABLE 1 - CLINICAL SYMPTOMS

PRODUCT: Amantilla Relax **PRODUCT ORIGEN: NutraMedix, LLC, Florida, USA**
DOSAGE: 20 ml/kg **SEX: Female**
START DATE: 04/22/05 **END DATE: 05/05/05**

CLINICAL SYMPTOMS	DAYS													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
EYES	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MUCOUS MEMBRANES	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RESPIRATORY SYS.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIRCULATORY SYS.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AUTONOMO	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CENTRAL NERV. SYS.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CHANGES IN HAIR	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TREMBLING	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CONVULSIONS	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SALIVATION	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SKIN	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SEDATION	+	-	-	-	-	-	-	-	-	-	-	-	-	-
SOMULENCE	+	-	-	-	-	-	-	-	-	-	-	-	-	-
DEATH	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OTHER	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PLEASE NOTE: THE NUMBER OF ANIMALS WITH THE SYMPTOM IS NOTED ON THE CHART.

TEC. RUNNING THE EXPERIMENT: **SIGNATURE:**
PROFESSIONAL RESPONSIBLE: MSc. Gastón García Simón **SIGNATURE:**

DATE DISSEMINATED: 05/05/05

Clinical symptoms were noted in the study group, as recorded on Table 1. Sedation and somnolence may be due to the alcoholic content of the preparation.

BODY WEIGHT:
Table 2 demonstrates the body weights of the animals on day 1, 7, and 14 of the experiment.

TABLE # 2. BODY WEIGHT (GRAMS) VARIATION AND STANDARD DEVIATION OF THE FEMALE ANIMALS IN THE EXPERIMENT OF ACUTE TOXICITY VIA ORAL INGESTION OF AMANTILLLA RELAX.

GROUP	TIME (Days)		
	0	7	14
FEMALES I	24.67 ± 1.15	25.0 ± 1.0	27.0 ± 1.0
FEMALES II	22.67 ± 1.15	23.0 ± 2.0	25.0 ± 1.0

As can be seen from Table 2, the females treated with Amantilla Relax showed weight gain between the weighing sessions.

The following graphs were created with the values from Table 1.

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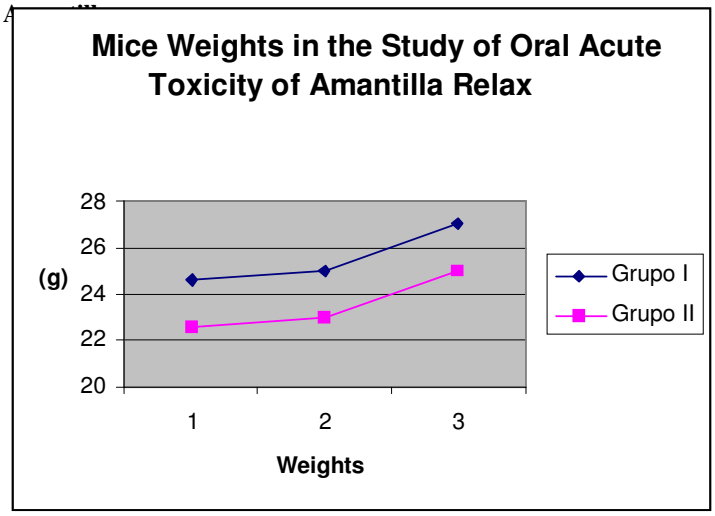

HISTO-PATHOLOGY:
Samples taken from selected organs showed no affects from under the microscope, thus the pathologist did not take histo-pathological samples.

CONCLUSIONS:

1- Clinical symptoms were observed in the animals, presumably due to the alcoholic content of the preparation.

2- Autopsies revealed no affects to selected organs.

3- The product did not affect weight gain of the animals in the study.

4- No toxic effects are produced when administering **Amantilla Relax** in an acute form to the animals.

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5- Amantilla Relax is a compound whose potential for toxicity is very low based on the fact that its security margin is very high. LD50* cannot be calculated because the maximum dose that can be administered to a mouse is 4 ml per 20 grams of body weight, the dosage used in this experiment. In addition, the dosage used in humans is diluted in 120 ml of water, unlike with the mice that received an undiluted dosage. All this means that, taking into account that the total dissolved solids (1.5 mg/ml) that were administered per 30mg of animal weight, and that humans consume only 6.5 x 10^{-4} mg, the innocuousness of the product is confirmed.

6- The LD50 of Amantilla Relax is much higher than the 30 mg/kg administered to the mice, which in turn is much higher than what humans receive, again confirming the safety of the product.

GENERAL CONCLUSIONS:

Amantilla Relax did not produce toxic effects when used in accordance to the guidelines described in OECD TG 423, thus the product is considered practically innocuous for humans when administered in the acute form, according to the Classification of the European Union labeled "Without Classification." Therefore studies of acute toxicity at higher doses in humans are not necessary.

PERSONNEL RESPONSIBLE FOR THE STUDY:

DIRECTOR OF THE STUDY: **SIGNATURE:**
DR. WALTER HERRERA:

Responsible Professional: **SIGNATURE:**
MSc. Gastón García Simón
Date: 05/05/05

* The dose at which 50 percent of the animals in the experiment suffer mortality.

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